

Bindung von Alkoholen an
die Peptid-CO-Gruppe von Poly-L-prolin

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Binding of Alcohols to the Peptide CO-Group of Poly-L-proline in the I and II Conformation.

I. Demonstration of the Binding by Infrared Spectroscopy and Optical Rotatory Dispersion

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Synopsis

The binding of *n*-butanol, benzyl alcohol, and trifluoroethanol to polyproline I (all peptide bonds in *cis* configuration) and II (all peptide bonds in *trans* configuration) has been demonstrated. It was found by infrared spectroscopy that hydrogen bonds are formed between the CO groups of the polymer and the OH groups of the alcohols. Their strength increases from *n*-butanol via benzyl alcohol to trifluoroethanol. The binding of benzyl alcohol could also be followed by optical rotatory dispersion due to the fact that the electronic transitions of the benzyl ring are rendered optically active by the binding to the polymer.

INTRODUCTION

Poly-L-proline exists in two conformational forms, I and II, the structures of which have been elucidated by x-ray crystallography.¹⁻³ Form I is a right-handed helix with all peptide bonds in the *cis* configuration, and form II is a left-handed helix with all peptide bonds in the *trans* configuration. Poly-L-proline does not possess NH groups, and therefore, no hydrogen bonds between peptide groups help to stabilize the helices. Steric effects, however, play a dominant role in the stabilisation because the conformational energy, which is predicted from the steric conditions alone,⁴ shows minima for the two known structures of the polyproline chain. From the simple steric considerations, which do not imply polymer solvent interactions, it follows that the form I may have intrinsically a slightly higher stability.⁴

The solvent determines which of the two types of helices is stable in solution. Form I, for example, is stable in pyridine and in unsubstituted aliphatic alcohols, and form II is stable in water, acetic acid, formic acid, benzyl alcohol, and trifluoroethanol.⁵⁻⁸ This shows that not only the steric conditions of the polyproline chains, but also the interactions with the solvent must play an important role in stabilizing the structures. A reversible transformation $I \rightleftharpoons II$ may be induced by appropriate changes

of the solvent system.⁵ Due to the cooperative nature of this process⁸⁻¹³ very small changes in the conditions which influence the stability of the two forms are sufficient to initiate the transition from one form to the other.

The simplest way of explaining a shift of the $I \rightleftharpoons II$ equilibrium toward II (I) is by a preferential binding of the solvent to II(I). The statistical thermodynamical formulation¹⁴ of this mechanism has been very successfully applied to describe the equilibrium curves of the solvent induced $I \rightleftharpoons II$ transition in benzyl alcohol-*n*-butanol and trifluoroethanol-*n*-butanol.¹⁵

A probable candidate for the site of interaction on the polymer is the CO group, which may form hydrogen bonds with the alcohols. Infrared spectroscopy was chosen as a method for detecting these hydrogen bonds. The studies were supplemented by ORD measurements in the case of benzyl alcohol, because it was expected that the electronic transitions of the benzyl ring would be rendered optically active on binding of this alcohol to the polymer.

The aim of our studies on the binding of alcohols to poly-L-proline is to prove or disprove the hypothesis¹⁵ that a preferential binding of alcohols to one of the two forms takes place. To this end spectroscopic effects had to be detected by which the relative binding constants of the alcohols to both forms could be measured in solution. This first part of our work shows qualitatively that a binding takes place and lays down the foundations for the measurements of the binding constants which will be reported in a following paper.¹⁶



EXPERIMENTAL

Materials

poly-L-proline with a weight-average molecular weight of 3200 was purchased from Miles Yeda Ltd., Rehovot, Israel. Other batches (molecular weights 6000, 9000, 10000) were synthesized by *N*-carboxy-L-proline anhydride polymerization¹⁷⁻¹⁹ in pyridine, acetonitrile, and methylene chloride, respectively. Weight-average molecular weights were determined by prior ultracentrifugation (Yantis midpoint method)²⁰ in 0.1M sodium chloride solution. The synthesized material was in form I as shown by its positive optical rotation ($[\alpha]_{360\text{ m}\mu} = +600^\circ$) in trifluoroethanol. Benzyl alcohol and *n*-butanol were of reagent grade (Merck, Darmstadt, Germany) and trifluoroethanol was purchased from Schuchardt, Munich, Germany. All solvents were extensively dried with molecular sieves (Merck, Poremasieb 3 Å).

Infrared Methods

An infrared cell suitable for vacuum work was built from stainless steel with windows made of germanium (thickness 3 mm, diameter 27 mm). The germanium was *n*-doped and of high purity (specific resistance >40

ohm-cm). One window was removable to permit casting of the polyproline films on its inner surface. A Teflon ring was placed around it in order to form a container with the window as the bottom.

Poly-L-proline, 3-5 mg, was dissolved in about 2 ml of trifluoroethanol, and the solution was poured on the window. The solvent was evaporated by application of vacuum with slow rotation of the window. The procedure took less than 5 min at 0°C. This was important for polyproline I which transforms into form II in trifluoroethanol. As a test for the fraction of form II in films of form I, a film prepared in this way was quickly dissolved in trifluoroethanol. From the initial value of optical rotation of this solution a fraction of 0.2 was determined. Similar estimates were obtained from a direct observation of the infrared band at 960 cm^{-1} in the film. This band is characteristic of form I. Because of the unknown thickness of the film, however, this method was less reliable than the measurement of initial optical rotation.

The reassembled cell was evacuated (10^{-2} mm Hg) until the infrared spectrum of the OH region no longer indicated traces of trifluoroethanol or water. An IR 12 spectrometer from Beckman Instruments, Fullerton, California, USA was used. A container with the alcohol, thermostated at 22°C (about 5°C lower than any other part of the system) was then connected to the cell. The vapor pressures were 50 mm Hg for trifluoroethanol, 7 mm for *n*-butanol, and 3 mm for benzyl alcohol. The reference cell was connected to the sample cell in order to compensate for the absorbance of the alcohol vapor. Spectra were recorded at time intervals of 12-24 hr. The spectra of the pure alcohols in the liquid state were measured by use of a commercial variable-path length cell equipped with Irtran 2 windows (Beckman Instruments, Fullerton, Calif., USA). The path length was about 25 μ .

Optical Rotatory Spectra

Due to the high absorption of benzyl alcohol a cell of short path length (Type UV-10, Beckman Instruments) was used with lead spacers of 7 μ thickness. The exact path length of the empty cell was determined from the distance between the periodically appearing small maxima in the absorption spectra, which were caused by interference of the direct beam with the beam reflected at the inner surfaces of the windows. No interference takes place in a completely filled cell, but small islands of air usually allow a path-length determination in this instance as well. The base line was measured with the empty cell because an exchange of solution in the assembled cell is not possible. This causes an uncertainty in the base-line determination of $\pm 0.025^\circ$ in the wavelength region of interest. The rotation of poly-L-proline in water was measured with 0.1 mm, 1 mm, and 10 mm silica cells (Opticell, 4202, 37th Street, Brentwood, Maryland 20722, USA and Bodenseewerk Perkin-Elmer, Überlingen, Germany). The optical rotatory dispersion was measured with a recording spectropolarimeter (Cary 60, Varian, Monrovia, Calif., USA).

RESULTS

After the polyproline films (I or II) are exposed to the alcohol vapor, changes in the infrared spectra occur which indicate a binding of the alcoholic hydroxyl group to the peptide carboxyl group. A shift of the amide I band (which is essentially caused by the CO stretching vibration), toward lower wave-numbers is observed. Simultaneously, the stretching vibration of the alcoholic OH group becomes apparent in the spectrum of the film (Fig. 1). No further changes of the spectrum are observed after 1 day for trifluoroethanol, after 2 days for *n*-butanol, and after 4 weeks for benzyl alcohol. We are not certain, however, whether for benzyl alcohol, which has an extremely small vapor pressure, a real equilibrium value was achieved. The size of the OH bands and therefore the amount of alcohol in the films are largest in the case of trifluoroethanol and smallest for benzyl alcohol. The size of the bands depends on the thickness of the film, on the affinity of binding, and on the vapor pressure. Therefore, no quantitative evaluation of their size has been made.

Amide I Region (CO-Stretching Vibration)

The amide I band shifts from its position of 1655 cm^{-1} to a position of about 1635 cm^{-1} when trifluoroethanol binds. The observed shift is

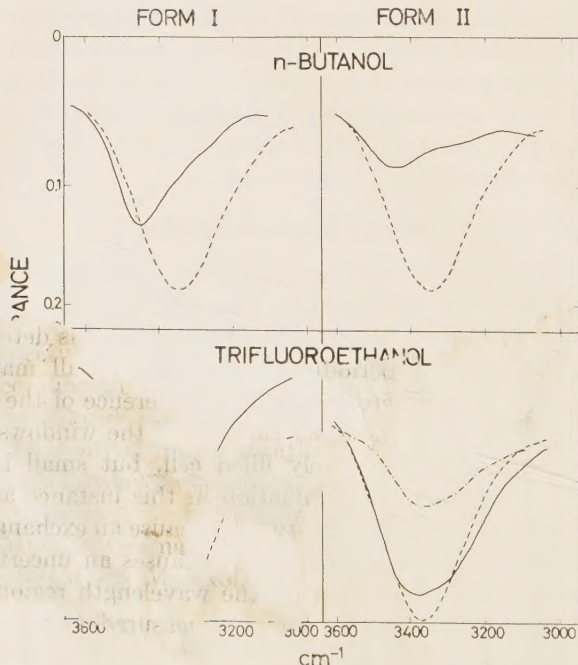


Fig. 1. OH-stretching band of *n*-butanol and trifluoroethanol hydrogen-bonded to films of poly-L-proline (—); (---) spectra of the pure alcohols shown for comparison; (-.-) spectrum recorded reduced vapor pressure (20 mm of Hg).

TABLE I

Position of the Amide I Band in Poly-L-proline Before and After Binding of Various Alcohols

Poly-L-proline	Wavenumber, cm^{-1}			
	Free	With <i>n</i> -butanol	With benzyl alcohol	With trifluoroethanol
I	1655	1650 ^a	1650 ^a	1635
II	1655	1650 ^a	1650 ^a	1635

^a These values are too large because of incomplete binding (see text).

approximately the same for form I and II. Its size and direction correspond to the shifts which are usually observed when the CO group forms a hydrogen bond.²¹ Table I shows that the displacement of the band is smaller for benzyl alcohol and *n*-butanol than for trifluoroethanol. It would be erroneous to deduce from this observation, however, that the hydrogen bonds formed by *n*-butanol and benzyl alcohol are weaker than those formed by trifluoroethanol, the amount of alcohol bound being very different in the three cases. It may be estimated from the size of the OH bands that only a small fraction of the CO groups is saturated in the cases of *n*-butanol and benzyl alcohol. In this instance the overlapping bands of the free and the bound CO give nearly the same picture as one slightly shifted band.

Region of the OH-Stretching Vibration

The band of the OH-stretching vibration of *n*-butanol (Fig. 1) and of benzyl alcohol (not shown) when bound to poly-L-proline always appears to be composed of two components which are not well separated. It is assumed that the main component, which does not agree in position with the OH-band of the liquid alcohol, (Table II) stems from the alcoholic hydroxyl groups bound to the CO groups.

TABLE II

Position of the Stretching Vibration of the Hydroxyl Groups in Various Alcohols Bound to Poly-L-proline and Reference Compounds ($T = 35^\circ\text{C}$)

Alcohol	Wavenumbers ν_{OH} , cm^{-1}		
	Liquid alcohol	Alcohol bound to	
		Polyproline I	Polyproline II
<i>n</i> -Butanol	3340 ± 10	3450 ± 10	3450 ± 10
Benzyl alcohol	3340 ± 10	3400 ± 20	3400 ± 20
Trifluoroethanol	3370 ± 10	3320 ± 70	3320 ± 70

For trifluoroethanol one broad band at approximately the same position as the band for the liquid alcohol is observed (Fig. 1). In this instance the OH band of the CO-bound alcohol may be given only with considerable uncertainty (Table II). Removal of part of the alcohol at a reduced vapor pressure did not result in a better resolution (Fig. 1).

The positions of the OH-stretching vibrations are summarized in Table II.

It is useful to express the data in terms of the difference between the OH-stretching vibration of the free alcohol molecules (in the gaseous state) and the observed band positions for the bound alcohols.²¹

$\Delta\nu_{\text{OH}} = \nu_{\text{free OH}} - \nu_{\text{bound OH}} = 3620 - \nu_{\text{bound OH}}$. The value of $\nu_{\text{free OH}}$ is nearly the same for all primary alcohols and the average value is about 3620 cm^{-1} .²² The $\Delta\nu_{\text{OH}}$ values increase from *n*-butanol to trifluoroethanol, (Table III), when these alcohols form hydrogen bonds with the CO groups of poly-L-proline. A survey of literature data (Table III), shows that this sequence is maintained for other acceptor groups. Literature values are available only for ethanol but little difference is expected to exist between the $\Delta\nu_{\text{OH}}$ of *n*-butanol and ethanol. Also, no complete set of $\Delta\nu_{\text{OH}}$ values for all three alcohols to one common acceptor was available from literature. Therefore, overlapping data from different acceptors were combined (Table III).

TABLE III
Position of OH Stretching Vibration in Terms of Difference

Alcohol	$\Delta\nu = 3620 - \nu_{\text{OH}}, \text{cm}^{-1}$				
	Polyproline		Acetone	Diisopropyl ether	Pyridine
	I	II			
<i>n</i> -Butanol (Ethanol)	170	170	(109) ^a	(164) ^b	(176) ^a
Benzyloethanol	220	220	125 ^c	—	315 ^d
Trifluoroethanol	300	300	166 ^e	251 ^f	—

^a 17

line data (last column) by $\Delta\nu = (311 - 276)/109$.

^d Seevogel.²⁵

y Dispersion

on wavelength has been measured for polyproline I and II in benzyl alcohol in Fig. 2). Polyproline II shows the negative part of a Cotton effect in this region,²⁷ with a minimum at $217 \text{ m}\mu$. In benzyl alcohol, on the other hand, a completely different dispersion curve is obtained. A number of small Cotton effects are present in the region of

245–270 $m\mu$. These seem to be related to the absorption bands characteristic of the benzene ring in the same region. Another extremely sharp Cotton effect is observed at about 227 $m\mu$ with a minimum at 230 $m\mu$, in a region where benzyl alcohol exhibits a strong absorption band. For polyproline I in benzyl alcohol this large Cotton effect appears again at 227 $m\mu$ but is opposite in sign. The smaller Cotton effects are situated at the same wavelengths as those of polyproline II, and their signs seem to be inverted. The amplitudes of the Cotton effects cannot be compared quantitatively because the spectrum of the pure form I could not be determined. At the time of measurement about 30% of the poly-L-proline was already converted into form II, which is the stable form in benzyl alcohol. Another uncertainty arises from the difficulty of an accurate determination of the baseline of the 7μ cell. The range of error involved in the baseline determination (see Experimental) leads to an uncertainty in the specific optical rotation of ± 1500 . The inaccuracy of the actual measurement is much lower and is indicated by the bars in Figure 2.

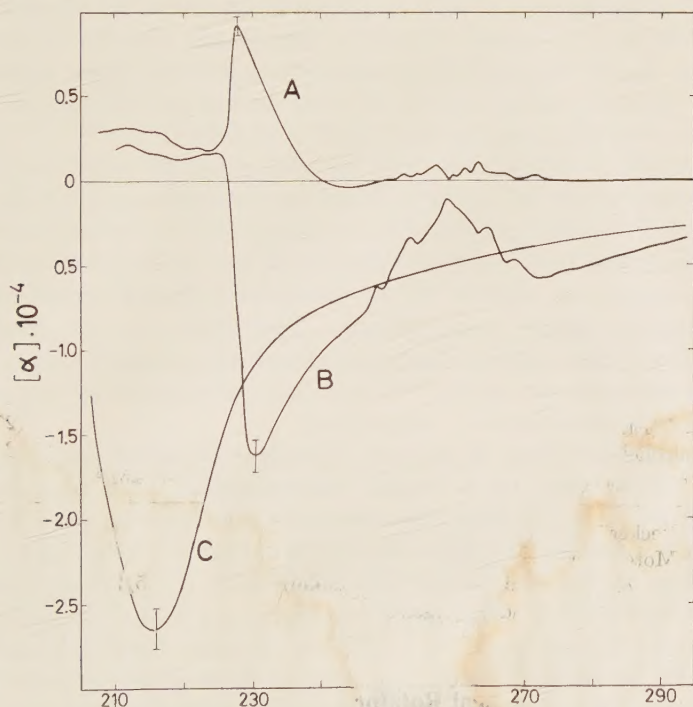


Fig. 2. Optical rotatory dispersion of polyproline I and II in benzyl alcohol: (A) polyproline I, $c = 5.0$ g/100 ml (molar ratio of proline residues to benzyl alcohol 1:18); (B) same solution after complete conversion to form II (all measurements in benzyl alcohol performed in a cell with a path length of about 7μ); (C) polyproline II in water, $c = 0.18$ g/100 ml, in a cell with a 0.1-mm path length. Poly-L-proline with a weight-average molecular weight of 3200 was used.

DISCUSSION

Our results show that all three of the alcohols investigated—benzyl alcohol, *n*-butanol, and trifluoroethanol—bind to polyproline I and II. This follows from the shift of the CO-stretching vibration band of poly-L-proline and is confirmed by the changes in the positions of the OH-stretching bands of the alcohols, both of which show up in the infrared. The binding could be exemplified, in addition, by ORD measurements in the case of benzyl alcohol. The ultraviolet absorption bands of this aromatic alcohol become optically active when it binds to poly-L-proline.

In regard to the nature of the binding, it may be deduced that hydrogen bonds are involved. The observed spectral shifts $\Delta\nu_{\text{OH}}$ correspond in direction and magnitude to the effects expected for the formation of this type of bond. The stretching vibration which stems from the OH groups bound to CO groups is always accompanied by a second band. This has a similar position as the OH stretching band in pure alcohol, in which the molecules are crosslinked by hydrogen bonds. A possible explanation of this band is the binding of further alcohol molecules to the alcohol which is bound to the CO group. This is in accordance with the observation that both bands disappear simultaneously when the vapor pressure is reduced. Alcohol molecules with free OH groups are not present in the film because no band around 3620 cm^{-1} is observed.

Two molecular explanations may be offered for the occurrence of extrinsic Cotton effects belonging to electronic transitions of the benzyl ring in the benzyl alcohol–poly-L-proline system: (1) the symmetric benzyl chromophore has been rendered asymmetric by placing it in a dissymmetric environment, and/or (2) an electronic coupling occurs between asymmetrically placed chromophores. The ORD or CD spectra of various biopolymers, which either contain aromatic groups^{28–32} or to which aromatic molecules are bound^{32–36} have been interpreted by one or both of the above explanations.

One requirement has to be met regardless of which explanation is correct. There must be a regular arrangement of the chromophores along the polymer. If they were randomly oriented with respect to the asymmetric moieties, no optical activity would arise. This together with the infrared results lead to the following picture of the benzyl alcohol–poly-L-proline system. The alcohol molecules are bound with their OH groups to the CO groups of poly-L-proline, thereby, forming a secondary helix. The interesting question of whether there is an electronic interaction between the alcohol molecules cannot be answered from the present spectroscopic data. It is tempting to postulate that the large and very sharp Cotton effect at $227\text{ m}\mu$ is caused by such interactions and therefore, measures the optical activity of this secondary helix. This seems to be supported by the observation that this Cotton effect changes its sign when the left-handed polyproline II helix is transformed into the right-handed I helix. One should, however, keep in mind that the direct asymmetric environment of the alcohol molecules may also be inverted

during this transformation, which would give rise to the same type of observation.

Some information on the relative strength of the hydrogen bonds involved in the binding may be derived from the $\Delta\nu_{\text{OH}}$ values which are a semiquantitative measure²¹ of the strength of hydrogen bonds. By this criterion, the strength increases from *n*-butanol to benzyl alcohol to trifluoroethanol. This sequence is in agreement with literature data on hydrogen bonds between the same alcohols to other acceptor groups (Table III).

The accuracy of our determination of the $\Delta\nu_{\text{OH}}$ values is not sufficient for detection of possible minor differences between the acceptor strengths of the CO groups in the form I and II of poly-L-proline (Table II). A demonstration of such a dependence of the strength of the hydrogen bonds on the conformation of the polymer would in any case not decide the question whether a preferential binding takes place. The binding constants in solution are determined not only by the strength of the hydrogen bonds but also by other stabilizing and destabilizing interactions and by entropic effects. Therefore equilibrium constants of the binding of the alcohols to poly-L-proline have been measured in solution.¹⁶ The ratio between the extinctions of the amide I bands of free and bound poly-L-proline has been used as a measure for the degree of binding. It is seen from the binding constants that trifluoroethanol binds better to polyproline II than to I. The binding constants to I or II increase from *n*-butanol to benzyl alcohol to trifluoroethanol. The values of the binding constants and a discussion of a possible molecular explanation of their differences will be published in the second part of this work.¹⁶

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Lebenslauf

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Die Reifeprüfung legte ich im Juni 1958 an der Rupprecht-Oberrealschule in München ab.

Im Wintersemester 1958/59 begann ich an der Ludwig-Maximilians-Universität in München das Studium der Physik. Hier legte ich im Wintersemester 1961/62 das Vordiplom und im Wintersemester 1966/67 die Diplom-Hauptprüfung ab.

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